DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
72-3(C)

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** Bifenthrin Purity: 93.6%

3. **CITATION**

Authors:	Fournier, A.E., *et.al.*
Title:	Bifenthrin - Acute Toxicity to Mysids (*Americamysis bahia*) under Flow-through Conditions, Following OCSPP Draft Guideline 850.1035
Study Completion Date:	February 18, 2013
Laboratory:	Smithers Viscient 790 Main Street Wareham, MA 02571-1037
Sponsor:	Consumer Specialty Products Association, Inc. For The Bifenthrin Task Force Steering Committee/Joint Venture
Laboratory Report ID:	14011.6115
MRID No.:	49060102
DP Barcode:	410227

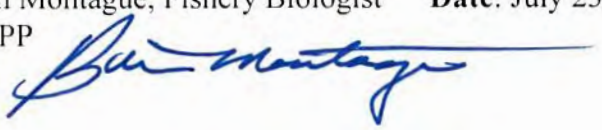
4. **REVIEWED BY:** David A. McEwen, Staff Scientist, CSS-Dynamac

Signature: David A. McEwen **Date:** 8/20/13

APPROVED BY: John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature: John Marton **Date:** 10/26/13

5. **APPROVED BY:** Brian Montague, Fishery Biologist **Date**: July 23, 2015
ERB5/OPP/EFED/OSCPP



6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external party for conducting this study or to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data

requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Age or Size of Test Organism:	Juveniles (≤24 hours old)
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

The LC_{50} value calculated in this study was 4.6 ng ai/L, categorizing Bifenthrin as **very highly toxic** to mysid shrimp on an acute toxicity basis.

Results Synopsis

96-hr LC_{50}: 4.45 ng ai/L 95% C.I.: 2.53-6.16 ng ai/L

Probit Slope: 3.86 (2.15-5.56)

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: This study complied with accepted test methods outlined in 850.1350 and is in general compliance with guideline requirements.

C. Reparability:

10. BACKGROUND

11. GUIDELINE DEVIATIONS

The study followed a protocol that was based on the procedures outlined in the US Environmental Protection Agency Series 850-Ecological Effects Test Guidelines, OCSPP Number 850.1035: *Mysid Acute Toxicity Test*. The following deviation from the OCSPP 850.1035 guidelines was noted:

1. The photoperiod used was 16 hours light/8 hours dark. A photoperiod of 14 hrs light/10 hrs dark is recommended.

2. Test Vessel size and test water volume were somewhat smaller than recommended, though flow rate and volume additions per day were adequate.

3. Oddly, test concentration measurements in the two higher concentration seemed to

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1. The photoperiod used was 16 hours light/8 hours dark. A photoperiod of 14 hrs light/10 hrs dark is recommended.

2. Test Vessel size and test water volume were somewhat smaller than recommended, though flow rate and volume additions per day were adequate.

3. Oddly, test concentration measurements in the two higher concentration seemed to

be reversed with the 13 ng ai/L nominal measuring a mean of 23 ng ai/L and the 25 ng ai/L nominal concentration measuring a mean of 14 ng ai/L. The laboratory has stated this may have been due to adsorptive properties of Bifenthrin. Two zero hour measured concentrations may have skewed the means in 2 of the concentrations. These two concentrations both resulted in 100% mortality and therefore do not define the LC50.

These deviations did not affect the validity of the study.

12. SUBMISSION PURPOSE: This study was submitted to provide information on the effects of Bifenthrin on mysid (*Americamysis bahia*) survival following acute exposure for the purpose of chemical reregistration (RR).

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Americamysis bahia, Penaeus setiferus, P. duorarun, P. aztecus* and *Palaemonetes sp.*	Saltwater mysid, *Americamysis bahia*
Age Juvenile, mysids should be < 24 hours old	Juvenile (≤24 hours)
Supplier	In-house cultures
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	2 weeks

Guideline Criteria	Reported Information
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Fed live brine shrimp nauplii (*Artemia salina*) up to twice daily prior to and during the test.
Pretest Mortality <3% mortality 48 hours prior to testing	None reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural seawater collected from the Cape Cod Canal (Bourne, MA) diluted to a salinity of 20±3‰ with laboratory well water and filtered (20 and 5 µm) prior to use.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17‰ for estuarine (euryhaline) shrimp, weekly range <6‰	20‰
Water Temperature Approx. 22±1 °C	24 to 25 °C

Guideline Criteria	Reported Information
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range <0.8	7.6 to 7.9
Dissolved Oxygen Static: ≥60% during 1st 48 hrs and ≥40% during 2nd 48 hrs, Flow-through: ≥60%	≥5.8 mg/L A DO concentration of 4.4 mg/L represents 60% saturation at 20‰ and 25°C
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	1.2 mg/L; during October 2012.
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms > 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), **3.9 L** is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms > 0.5 g, **2-3 L** is acceptable for smaller organisms.	1) Glass battery jars 2) 1.6 L 3) 1.4 L (15 cm depth)
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter. Delivery of the test solutions was initiated 4 days prior to the start of the study in order to achieve equilibrium.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	*Ca*. 6 volume additions/day
Biomass Loading Rate Static: < 0.8 g/L at < 17°C, < 0.5 g/L at >17°C; flow-through: < 1 g/L/day (N/A for mysids)	N/A

Guideline Criteria	Reported Information
Photoperiod 16 hours light, 8 hours dark	16 hr light, 8 hr dark, with transition periods. Light intensity was 330-530 lux.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Acetone; 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	Nominal test concentrations were selected based on the results of a 96-hr preliminary acute test under static conditions. Both neonate (≤24 hr old, Replicate A) and juvenile (5-6 days old, Replicate B) mysids were exposed to nominal concentrations of 0 (negative and solvent controls), 1.6, 3.1, 6.3, 13, and 25 ng ai/L. Based on more toxicity (mortality) observed in the neonate groups and in consultation with the Sponsor, neonate mysids were selected for use in the definitive test.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative and solvent controls), 1.6, 3.1, 6.3, 13, and 25 ng ai/L generally represent less than a 50% increase
Number of Test Organisms Minimum 20/level, may be divided among containers	20/level (10/replicate)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1) Temperature was measured in each test chamber daily. The temperature was also continuously measured in Replicate A of the 6.3 ng/L treatment level. 2) The dissolved oxygen concentration and pH of each test solution were measured daily.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if a flow-through system was used.	The concentration of the test material in the test solutions was measured at approximately 0, 48, and 96 hours using gas chromatography with mass selective detection (GC/MSD).

14. REPORTED RESULTS

A. General Results

ideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated Data Confidentiality, Quality Assurance, and GLP compliance statements were provided. The study was conducted in accordance with U.S. EPA GLP standards (40 CFR part 160), with one exception: periodic analyses of saltwater for potential contaminants were not performed according to GLP, but were performed in a certified laboratory using standard US EPA analytical methods.
Recovery of Chemical	50-170% of nominal from test samples; 82.2-99.5% of nominal in the QC samples
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	5 and 0% in the negative and solvent controls, respectively.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality [a]

Concentration (ng ai/L)		Number of Shrimp	Cumulative Number Dead (% mortality)			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Neg. Control	<LOQ [b]	20	1 (5%)	1 (5%)	1 (5%)	1 (5%)
Solv. Control	<LOQ [b]	20	0	0	0	0
1.6	0.79	20	0	1 (5%)	1 (5%)	1 (5%)
3.1	1.9	20	1 (5%)	1 (5%)	1 (5%)	3 (15%)
6.3	8.2	20	2 (10%)	5 (25%)	12 (60%)	16 (80%)
13	23	20	0	17 (85%)	20 (100%)	20 (100%)
25	14	20	3 (15%)	20 (100%)	20 (100%)	20 (100%)

a Data were obtained from Table 3 on page 26 of the study report.
b LOQ = 0.56 ng ai/L

Other Significant Results:

With the exception of 2 mysids appearing lethargic in the mean-measured 8.2 ng ai/L group, all surviving mysids appeared normal.

B. Statistical Results

Statistical Method: The LC_{50} values and 95% confidence intervals were calculated using CETIS™ statistical software. The NOAEC value was determined by visual interpretation of the mortality data.

96-hr LC_{50}: 4.6 ng ai/L 95% C.I.: 0.47 to 6.2 ng ai/L
NOAEC: <0.79 ng ai/L
Probit Slope: not reported

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
48-Hours	
Probit LC_{50} (95% C.I.)	10.4 (8.64-12) ng ai/L
Probit Slope	8.03 (4.21 to 11.9)
Trimmed Spearman Karber (95% CI)	9.42 (7.71-11.5) ng ai/L
96-Hours	
Probit LC_{50} (95% C.I.)	4.45 (2.53-6.16) ng ai/L
Probit Slope	3.86 (2.15 to 5.56)
Trimmed Spearman Karber (95% CI)	4.31 (3.38-5.49) ng ai/L

16. REVIEWER'S COMMENTS:

After 96 hours, mortality averaged 5% and 0% in the negative and solvent controls, respectively, and 5, 15, 80, 100, and 100% in the mean-measured 0.79, 1.9, 8.2, 23, and 14 ng ai/L treatment groups, respectively. With the exception of 2 mysids appearing lethargic at the 96-hr interval in the mean-measured 8.2 ng ai/L group, all surviving mysids appeared normal.

The reviewer is somewhat concerned that test concentration measurements for day 0 appear to have reflected a possible spike in test material or perhaps an error in sampling for the 13 ng ai/L nominal test concentration. Also recoveries for the highest test concentration (25 ng ai/L) are only 56% of the nominal which appears low in comparison to the next lower concentrations (even with the omission of the day zero measurement in the 13 ng ai/L concentration). This discrepancy is not reflected in the quality control sample for the 25 ng ai/L sample and thus may reflect some adsorption of test material to test vessels or organic matter within the system. The two highest concentration both produced 100% mortality and thus, should not have affected the LC50 determination.

The in-life phase of the definitive test was performed from October 19-23, 2012.

17. REFERENCES: None; other than standard guidelines and methodologies.

CETIS Summary Report

OPPTS 850.1035 Acute Invert (Mysid)

Laboratory- Smithers Viscient

Batch ID:	03-3310-4493	**Test Type:**	Mortality (96-h)	**Analyst:**	
Ending Date:	12 Jul-12	**Protocol:**	OPPTS 850.1035 Acute Invert (Mysid	**Diluent:**	Seawater
	NA	**Species:**	Mysidopsis	**Brine : Age:**	Not Applicable
		Source:	bahia Lab In-		<24h
Sample ID:	09-3654-6571	**Code:**	49060102	**Client:**	CDM Smith
Sample Date:	12 Jul-12	**Material:**	Bifenthrin	**Project:**	Insecticide
Receive	NA	**Source:**	Bifenthrin Task Force Steering Committee/		
		Station:			

Batch Note: PC Code 128825 MRID 49060102

Sample Note: PC Code 128825 MRID 49060102

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-0287-3674	48h Mortality Rate	0	>0		NA		Fisher Exact Test
06-3444-8672	96h Mortality Rate	0	>0		NA		Fisher Exact Test

Point Estimate Summary

Analysis ID	Endpoint	Level	ng ai/L	95% LCL	95% UCL	TU	Method
14-8379-6665	48h Mortality Rate	LC5	6.48	3.84	7.99		Linear Regression (MLE)
		LC10	7.19	4.65	8.65		
		LC15	7.72	5.28	9.13		
		LC20	8.16	5.83	9.56		
		LC25	8.56	6.33	9.95		
		LC40	9.66	7.74	11.1		
		LC50	10.4	8.64	12		
21-1561-8139	48h Mortality Rate	LC50	9.42	7.71	11.5		Spearman-Kärber
06-5349-0861	96h Mortality Rate	LC5	1.67	0.479	2.82		Linear Regression (MLE)
		LC10	2.07	0.7	3.31		
		LC15	2.4	0.902	3.7		
		LC20	2.69	1.1	4.05		
		LC25	2.98	1.3	4.38		
		LC40	3.83	1.98	5.39		
		LC50	4.45	2.53	6.16		
17-6042-3239	96h Mortality Rate	LC50	4.31	3.38	5.49		Spearman-Kärber

48h Mortality Rate Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	1	0			0	0	0	0		
0	Negative Control	1	0.05			0.05	0.05	0	0	0.0%	
0.79		1	0.05			0.05	0.05	0	0	0.0%	
1.9		1	0.05			0.05	0.05	0	0	0.0%	
8.2		1	0.25			0.25	0.25	0	0	0.0%	
14		1	0.85			0.85	0.85	0	0	0.0%	
23		1	1			1	1	0	0	0.0%	

96h Mortality Rate Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	1	0			0	0	0	0		
0	Negative Control	1	0.05			0.05	0.05	0	0	0.0%	

0.79	1	0.05	0.05	0.05	0	0	0.0%
1.9	1	0.15	0.15	0.15	0	0	0.0%
8.2	1	0.8	0.8	0.8	0	0	0.0%
14	1	1	1	1	0	0	0.0%
23	1	1	1	1	0	0	0.0%

OPPTS 850.1035 Acute Invert (Mysid) **Smithers Viscient**

48h Mortality Rate Detail

C-ng ai/L	Control Type	Rep 1
0	Solvent Blank	0
0	Negative Control	0.05
0.79		0.05
1.9		0.05
8.2		0.25
14		0.85
23		1

96h Mortality Rate Detail

C-ng ai/L	Control Type	Rep 1
0	Solvent Blank	0
0	Negative Control	0.05
0.79		0.05
1.9		0.15
8.2		0.8
14		1
23		1

Concentrations of bifenthrin measured in the exposure solutions during the 96- flow through exposure of mysids *(Americamysis bahia).*

Nominal (ng/L)	Measured Concentration (ng/L) O hour	48-Hour	96 Hour	MeanMeasured"	Nominal (%)"
Control	<0.56b	<0.52	<0.54	NA°	NA
Solvent Control	<0.56	<0.52	<0.54	NA	NA
1.6	0.64	0.69	1.1	0.79	50
3.1	2.1	2.1	1.6	1.9	63
6.3	12	8.2	3.8	8.2	130
13	4ld	14	13	23d	170
25	16	14	13	14	56
QCe#l 1.00	0.930 (93.0)	0.913 (91.3)	0.822 (82.2)		
QC#2 6.00	5.08 (84.7)	5.02 (83.7)	5.48 (91.3)		
QC#3 25.0	20.6 (82.4)	23.6 (94.6)	24.9 (99.5)		

Mean measured concentrations and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) values presented in this table.

b Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the linear regression, the area of the low standards and the dilution factor of the controls.

NA = Not Applicable.

d This concentration overlaps the next higher concentration however was not used in determination of the LC50. (see Section 3.2.2).

QC = Quality Control sample with percent of recovery presented in parentheses .

Table 3.

Mean measured concentrations tested, corresponding mortalities and observations made during the 96-hour flow through exposure of mysids *(Americamysis bahia)* to bifenthrin.

Measured Concentration	24 Hour			48 Hour			72 Hour			96 Hour		
(ng/L)	A	B	Mea	A	B	Mean	A	B	Mean	A	B	Mean
Control	10 (1)	0 (0)	5	10 (1)	0 (0)	5	10 (1)	0 (0)	5	10 (1)	0 (0)	
Solvent Control	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
0.79	0 (0)	0 (0)	0	0 (0)	10 (1)	5	0 (0)	10 (1)	5	0 (0)	10 (1)	5
1.9	10 (1)	0 (0)	5	10 (1)	0 (0)	5	10 (1)	0 (0)	5	30 (3)	0 (0)	15
8.2	20 (2)	0 (0)	10	30 (3)	20 (2)	25	60 (6)	60 (6)	60	80 (8)	80 (8)	SOC
23	0 (0)	0 (0)	0	80 (8)	90 (9)	85	100 (10)	100 (10)	100	100 (10)	100 (10)	100
14	20 (2)	10 (1)	15	100 (10)	100 (10)	100	100 (10)	100 (10)	100	100 (10)	100 (10)	100
	(2)	(1)		(10)	(10)		(10)	(10)		(10)	(10)	

The actual number of mortalities is presented in parentheses.

[b]Response less than or equal to 10% is allowable in a control population and is considered within the expected range of naturally occurring variability (ASTM, 2002). Two mysids were observed to be lethargic.

The water quality parameters measured during the 96-hour flow through toxicity test exposing mysids (Americamysis bahia) to bifenthrin.

Nominal Concentration Hour (ng/L)	0-Hour		24-Hour		48-Hour		72-Hour		96-	
	A	B	A	B	A	B	A	B	A	B
					pH					
Control	7.9	7.9	7.8	7.9	7.8	7.8	7.8	7.8	7.8	7.8
Solvent Control	7.9	7.9	7.9	7.8	7.7	7.7	7.7	7.7	7.6	7.6
1.6	7.9	7.9	7.9	7.9	7.8	7.8	7.7	7.7	7.7	7.7
3.1	7.9	7.9	7.9	7.9	7.8	7.8	7.7	7.7	7.6	7.7
6.3	7.9	7.9	7.9	7.9	7.7	7.7	7.7	7.7	7.6	7.7
13	7.9	7.9	7.9	7.9	7.7	7.8	7.7	7.7	NAC	NAC
25	7.9	7.9	7.9	7.9	7.8	7.8	NA'	NA'	NA'	NAC
					Dissolved Oxygen (mg/L)"					
Control	7.5	7.6	7.9	7.9	8.0	8.0	7.2	7.2	7.4	7.4
Solvent Control	7.5	7.5	7.9	7.8	7.6	7.7	6.2	6.2	5.8	5.8
1.6	7.5	7.5	7.8	7.8	7.8	7.8	6.5	6.5	6.2	6.3
3.1	7.5	7.5	7.9	7.9	7.8	7.7	6.3	6.4	6.0	6.4
6.3	7.6	7.5	7.8	7.8	7.7	7.8	6.1	6.5	5.8	6.3
13	7.5	7.5	7.8	7.9	7.6	7.7	6.2	6.2 '	NA'	NA'
25	7.6	7.5	8.1	7.9	8.0	7.8	NAC	NA'	NAC	NAC
					Temperature (°C)b					
	24		25		24		25		25	
					Salinity (%0)					
	20		20		20		20		20	

60% of dissolved oxygen saturation value at 24 °C with a salinity of 20%0 is 4.5 mg/L. 60% of dissolved oxygen saturation value at 25 •cwith a salinity of 20%0 is 4.4 mg/L.

Values presented represent the measurement for all treatment level and control solutions at the stated time interval.

Continuous temperature monitoring of replicate A of the 6.3 ng/L (nominal) solution using a VWR minimum/maximum thermometer established a test solution temperature range of 24 to 25 °C during the exposure period.

[c] NA = Not Applicable. Water quality parameters were not measured due to 100% mortality observed at the previous interval.